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Quality Control in Mu DNA Transposition

Minireview

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Transposons are segments of DNA encoding protein factors that move the DNA segment to new locations in the genome. They are ubiquitous among organisms and play an important role in genome evolution, including the horizontal transfer of genetic information between species. Retroviruses may also be considered as transposons that possess a specialized mechanism to transfer their genome from one cell to another.

Considerable progress has been made in understanding the biochemical reactions that promote these DNA rearrangements (reviewed in Mizuuchi, 1992; Craig 1995; Lavoie and Chaconas, 1995). In some cases, the transposon is simply cut out from its original location and reinserted at a new site. Other transposons move by a replicative mechanism that leaves a copy of the transposon at the original location. It is clear that the basic chemical mechanisms that cleave DNA at the transposon ends and insert these ends in new locations in the genome are conserved among transposons in both prokaryotes and eukaryotes, and among retroviruses and retrotransposons. Recent studies suggest that the first stage of immunoglobulin gene rearrangement also occurs through a chemically similar mechanism (van Gent et al., 1996). Although the DNA cutting and joining mechanisms are similar among these diverse elements, each system has its own highly specific requirements for how these reactions must be temporally and spatially coordinated. Two reports in this issue of *Cell* (Aldaz et al., 1996; Savilahti and Mizuuchi, 1996) and one in press (Watson and Chaconas, 1996) highlight the role of an intricate nucleoprotein architecture in orchestrating the Mu transposition reaction.

Outline of Mu Transposition

Mu transposition proceeds through a series of stable nucleoprotein complexes, collectively called transpososomes. Three binding sites for transposase are located at each end of the Mu genome (Figure 1); these sites are designated L1, L2, and L3 at the Mu left end and R1, R2, and R3 at the Mu right end. In the absence of additional cofactors, transposase binds reversibly to each of these sites as a monomer. In the presence of the host encoded DNA bending protein HU, a divalent metal ion, and supercoiled Mu DNA, a Stable Synaptic Complex (SSC, also called Type 0 complex) is formed. In this complex, the two Mu ends are stably bound to a tetramer of transposase (Figure 2B). Formation of this stable complex also requires the participation of another DNA site, the Internal Activation Sequence (IAS), located about 1 kb from the left end of the Mu DNA; the reaction is also stimulated by another host encoded protein, IHF. The next step in the transposition pathway is a pair of single strand cleavages at the 3′ ends of the Mu DNA. The cleaved Mu DNA remains stably associated with the same tetramer of MuA protein in a complex called the Cleaved Donor Complex (CDC, also called Type 1 complex) as shown in Figure 2C. The Mu genome is then inserted into its new location in host DNA by a pair of transesterification reactions (DNA strand transfer) in which the hydroxyls at the 3′ ends of the Mu DNA attack a pair of phosphodiester bonds in the target DNA. In the resulting Strand Transfer Complex (STC, also called Type 2 complex) , the 5′ ends of Mu DNA are still joined to old flanking DNA sequence, while the 3′ ends of Mu DNA are joined to new flanking sequence (Figure 2D). This transposition intermediate can be resolved by assembly of replication forks at the three-way junctions, followed by replication through the Mu sequence to form a product called a cointegrate. Alternatively, nucleolytic cleavage can sever Mu DNA from the old flanking DNA in the transposition intermediate, and the single strand gaps between Mu DNA and flanking DNA are then repaired to generate a simple insertion transposition product.

The IAS Checkpoint

Formation of the first stable complex between Mu transposase and the Mu ends requires a sequence called the IAS or enhancer, that is located internal to the Mu left end (Figure 1). The IAS is an especially important regulatory element because it is involved immediately prior to formation of the SSC, an essentially irreversible step that represents commitment to transposition. Mu transposase binds to the IAS through a different domain from that involved in binding the sites at the ends of Mu DNA. The Mu transcriptional repressor protein blocks binding of Mu transposase to the IAS because the IAS overlaps the operator sequence to which repressor binds. Thus Mu repressor can down-regulate transposition at two levels; any transposase made prior to establishment of repression, or residual transposase expressed during lysogenic growth, must compete with repressor to bind the IAS. This dual system has presumably evolved to facilitate tighter regulation of transposition. Although the IAS is required to form the SSC, the IAS is not physically associated with the SSC once it has been made. Therefore, a three site complex involving the two ends of Mu DNA and the IAS has been inferred to be a transient intermediate in assembly of the SSC. The report from Watson and Chaconas (1996) provides the first direct physical evidence for the existence of this complex, which they call LER (Figure 2A). Although this complex had not previously been directly detected, Watson and Chaconas (1996) found that by elevating the concentration of transposase, and adding a cross-linking reagent at early time points, this complex could be trapped.

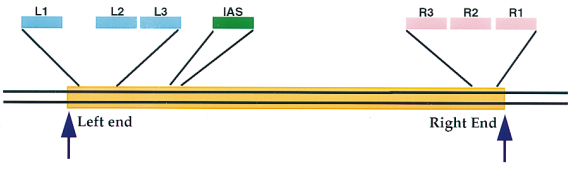

Figure 1. Mu DNA Sites Directly Involved in Transposition

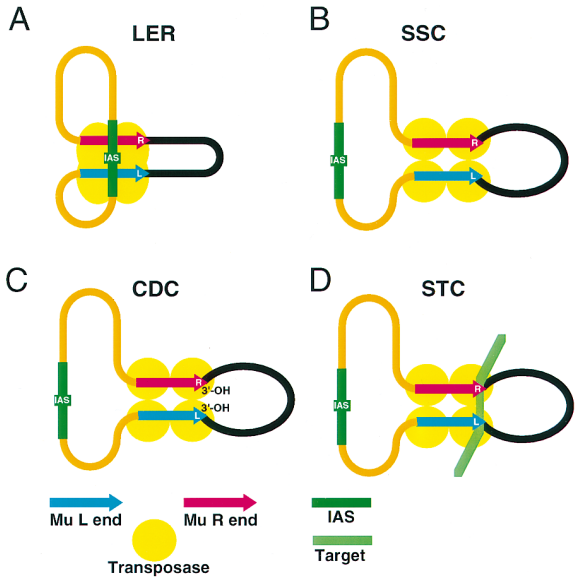

Figure 2. Nucleoprotein Complexes on the Mu Transposition Reaction Pathway.

The earliest complex detected to date is the three-site LER complex described in a report from Watson and Chaconas (1996). A global conformational change in the transposase multimer, depicted in yellow, results in the formation of the first stable transpososome, the SSC, in which a pair a Mu DNA ends are stably synapsed with a tetramer of transposase. Cleavage at the 3′ ends of the Mu DNA then generates the CDC. Finally, a pair of transesterification reactions insert Mu ends into target DNA to generate the STC.

Analysis by both electrophoretic methods and electron microscopy confirmed the presence of both the Mu left and right ends and the IAS in the complex.

Chaconas and coworkers previously showed that single base substitutions at the termini of Mu DNA inhibit formation of the SSC. The same base substitutions result in an accumulation of LER complexes, indicating that the block occurs after formation of the three-site LER complex. Conversion of the LER complex to the SSC is therefore likely to involve a global conformational change in the transposase multimer that brings the Mu DNA termini into close proximity with the active sites. Further evidence for major structural changes between the LER complex and stable transpososomes comes from the failure of transpososomes to trap the IAS in parallel experiments. The isolation of the LER complex as a physical entity, albeit after protein crosslinking, opens the possibility of studying the structure of this early transposition intermediate and the structural transitions that are involved in assembly of catalytically active transpososomes.

Mu Transposase Executes Cleavage and Strand Transfer in Trans

The reports from Aldaz et al. (1996) and Savilahti and Mizuuchi (1996) show that Mu A subunits do not catalyze the chemical steps of cleavage and strand transfer adjacent to DNA sites to which they are bound, but rather the active site from a bound subunit reaches across the transpososome and catalyzes the chemical steps in trans adjacent to DNA sites that are bound to different subunits (Figure 3).

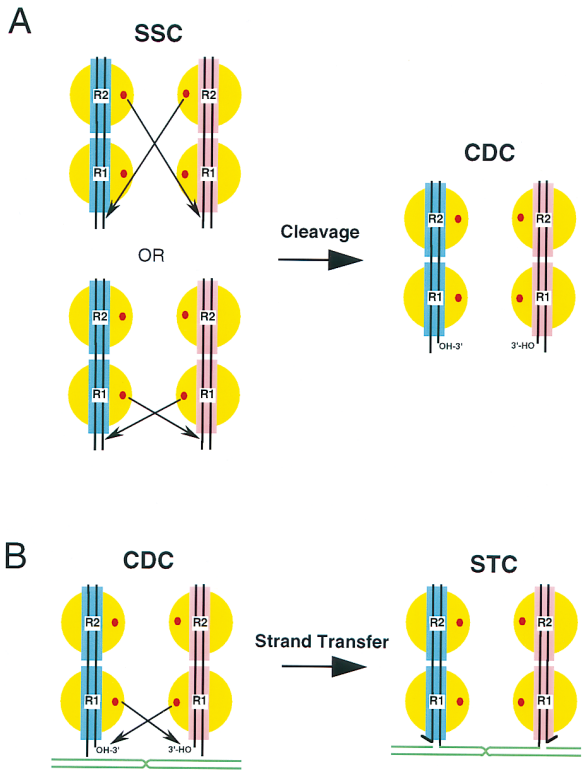

Figure 3. Trans Cleavage and DNA Strand Transfer within the Mu Transpososomes

(A) The stable synaptic complex involving a pair of modified Mu right ends, containing the transposase binding sites R1 and R2, and a tetramer of Mu transposase (yellow circles). The small red circles on the transposase subunits represent active sites. The tails of the arrows indicate the active site that is responsible for cleavage at the DNA sites to which the arrow heads point. The two patterns of cleavage that are consistent with the current data are shown.

(B) The active sites of the transposase bound to R1 catalyze DNA strand transfer adjacent to the R1 site of partner Mu DNA end. The target DNA for strand transfer is shown in green.

To correlate the sites of binding with the sites of chemical catalysis both groups made use of a simplified reaction system for in vitro Mu transposition. Instead of the normal pair of a left and right end of Mu DNA, a pair of simplified right end substrates was used. This reaction system bypasses the need for the IAS, but maintains the requirement for assembly into the SSC before any chemical activity takes place. Savilahti and Mizuuchi (1996) preloaded catalytically active transposase onto a Mu end DNA substrate, and catalytically inactive transposase onto a DNA substrate of different length. They then mixed the two preloaded Mu DNA ends and allowed SSC assembly to occur. The SSCs containing a short Mu end paired with a long Mu end were then isolated by gel electrophoresis and incubated with Mg^{2+} to allow cleavage to take place. Analysis of the resulting cleavage products revealed that when active transposase was preloaded onto the shorter Mu end, most of the cleaved Mu end DNA in the CDC was the longer Mu end, while most of the shorter Mu end DNA was not cut. When active transposase was preloaded onto the longer Mu end DNA, the bias was reversed and mostly the

shorter DNA in the CDC was cut. Similar experiments were carried out for strand transfer by analyzing whether the shorter or longer Mu DNA ends in the STC were joined to target DNA when one of the ends was preloaded with catalytically inactive protein and the other with active protein. The results demonstrate that the DNA strand transfer reaction is also catalyzed in trans. The active site of a monomer bound to one end of Mu DNA promotes strand transfer of the other Mu DNA end.

The report from Aldaz et al. (1996) tackles the same issue with a different experimental approach. Simplified Mu right end DNA substrates were again used, but the transposase binding site closest to the end (R1), or the internal R2 site, contained a short patch of ^{32}P-labeled DNA strand that was also derivatized with 5-Iodouracil to enable transposase to be crosslinked by irradiation with UV light. The reactions to make the STC included a mixture of active transposase and catalytically inactive transposase; the active transposase was truncated in a domain that is not required in the simplified reaction system so that it could be simply distinguished from inactive transposase by its electrophoretic mobility. STCs were isolated after UV irradiation from reactions that included Mu end DNA substrate with the labeled patch at either the R1 or the R2 site. The active transposase within the STC was preferentially crosslinked to the labeled patch (relative to the proportion of active to inactive transposase in the reaction mixture) only when the patch was at the R1 site. This result showed that active transposase must be bound to R1 for strand transfer to occur. The next question addressed in the report is whether transposase bound at R1 catalyzes strand transfer of the substrate molecule to which it is bound or the partner Mu DNA end. Reactions were carried out with pairwise combinations of substrate DNAs in which the labeled patch was either at the R1 site of a "good" Mu DNA end substrate or at the R1 site of a "crippled" substrate that is unable to carry out strand transfer because it lacks the 3′ adenosine that is joined to target DNA in the DNA strand transfer reaction. When the labeled patch was located at the R1 site of the crippled substrate, essentially all the crosslinked transposase in the STC was the active protein. This bias was not observed when the patch was at the R1 site of the good substrate. Therefore transposase bound to the R1 site of one Mu DNA end catalyzes DNA strand transfer of the partner Mu DNA end.

The results from the two reports are consistent with the model depicted in Figure 3. DNA strand transfer occurs in trans, with transposase bound to one R1 site donating its active site to join the partner Mu DNA end in the transpososome. Cleavage is also in trans, but neither paper addresses the question of whether the subunit donating the active site for cleavage is bound to the R1 or to the R2 site of the partner.

Nucleoprotein Architecture in Mu Transposition

A striking feature of the Mu transposition machinery is the utilization of nucleoprotein architecture as a regulatory mechanism. Assembly of the SSC, the first stable complex on the reaction pathway, is dependent upon transient formation of a three-DNA-site complex involving a pair of Mu DNA ends and the IAS. Mu repressor can directly block initiation of Mu transposition at this step because the binding site for Mu transposase in the IAS overlaps with the operator site to which repressor binds. Formation of the SSC as a prerequisite to cleavage at the ends of Mu DNA ensures that when one end is cleaved, the other end is also correctly positioned for cleavage.

The results from the Baker and Mizuuchi laboratories provide at least one reason why assembly of the proper complex must precede the chemical steps of cleavage at the ends of Mu DNA and strand transfer. Donation of active sites from subunits bound to the other end of Mu DNA requires that these steps can only occur after the SSC has formed. The result is to coordinate cleavage at the two ends of the Mu genome. Maintenance of a stable complex after the cleavage step ensures that the ends are correctly positioned for the strand transfer step, which requires coordinated joining of the two Mu ends to the target DNA to make a meaningful product. As in the case of the cleavage step, donation of the active sites for strand transfer from a subunit bound to the other end of Mu DNA serves to enhance the fidelity of the reaction.

Common and Divergent Themes

The emerging picture of DNA transposition is one of both striking similarities and differences among elements. On the biochemical level it is clear that the transposition mechanism is highly conserved. In all cases, transposition is initiated by cleavage at the 3′ ends of the transposon, and a transesterification reaction splices these ends into a target DNA. The mechanistic commonality is highlighted by the similarity of structures of the catalytic domain of Mu transposase, HIV-1 integrase, and ASV integrase (reviewed in Rice et al., 1996). Although the Mu transposase and retroviral integrases share very little common primary sequence, the three dimensional structures of the catalytic domain are very similar. We may safely conclude that the mechanism of chemical catalysis is highly conserved.

Although the series of DNA cutting and joining events are very similar among transposons, the transposase proteins themselves and their DNA sites for recognition are quite diverse in organization. Many transposons have a single transposase binding site at their termini, others like Mu and Tn 7 have a complex array of sites. In the case of Tn 7, transposase consists of at least four different polypeptides. Retroviral integrases appear to rely on the nucleoprotein structure of the viral core to assemble on the viral DNA ends. Unlike typical transposases, their binding affinity for viral DNA ends is not significantly greater than that for nonspecific DNA.

Transposons and their cousins have clearly evolved different strategies to suit their individual lifestyles. Although the nucleoprotein architecture of many related elements clearly can not be identical to Mu because the building blocks are not conserved, some features of the Mu architectural regulation may be common among transposable genetic elements. The requirement for a pair of DNA sites to undergo synapsis before the protein factors can be correctly positioned for catalysis provides an effective way of avoiding unproductive or abortive recombination events. Cooperation among protein monomers to form a catalytic unit may be a common mechanism to ensure that DNA cleavage and ligation

reactions are properly coordinated, as has been proposed for Flp recombinase (Chen et al., 1992) and Mu transposase (Yang et al., 1995).

Mu transposition must occur with extraordinarily high efficiency and fidelity during lytic growth, yet it must be totally shut down during lysogeny. The sophisticated regulatory system revealed by recent studies may have resulted from these disparate selection pressures operating at different stages of the replication cycle. It is a paramount example of the use of specialized nucleoprotein structures to regulate biochemical reactions with high precision, as discussed by Echols (1986).

Selected Reading

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Note Added in Proof

A report accepted for publication after completion of this minireview (J.-Y. Yang et al., 1996, Cell, in press) addresses matters closely related to those discussed in the papers from the Baker and Mizuuchi groups. The results of the study by Yang et al. also suggest that the Mu A monomer that donates the active site for cleavage is bound to the R2 site of the partner Mu DNA end, an issue not addressed in the other papers discussed here.